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NE 489 Nano-Biomaterials
Term Project Paper
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Engineering Nanomaterial Surfaces for Biomedical Applications

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1 Overview

Owing to their unique optical, electronic, magnetic, mechanical and chemical properties, an extensive amount of research has been done on nanomaterials. These advantageous properties can be attributed solely to their special size and shape. Furthermore, nanomaterials can be very easily functionalized on the surface with synthetic ligands to effect significant change in properties depending on the application at hand. In this respect, nanomaterials are very versatile and simple to use.

Nanomaterials have proven themselves to have a significant range of application in a variety of aspects of biology and medicine. Applications involving nanomaterials include (but not limited to) bio-molecular sensing, biological imaging, drug delivery vehicles in the form of micelles and vesicles, disease therapy, and as scaffolds in tissue engineering applications.

The most critical part of nanomaterials that makes them special is their surface. It is the surface that makes the nanomaterials significantly more useful than conventional non-nano materials. As the size of the material decreases, its surface-to-volume ratio increases.

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This presents considerable advantage to modify properties of nanomaterials through surface functionalization techniques.

There are several different categories of nanomaterials. Not surprisingly, the field of nanomaterials is quite vast, diverse and evolving rapidly as tremendous amounts of research is being conducted in this burgeoning area. In this paper, we will focus exclusively on classes of nanomaterials that are biomedically important. These materials are metals, semiconductors and nanotubes. The four important biomedically important classes of materials are:

1. *Metal Nanoparticles*

   Examples of metal nanoparticles are gold and silver nanoparticles. Metal nanoparticles are easy to prepare and quite stable even in unfavourable conditions. They also have unique optoelectronic properties that can be exploited for imaging applications

2. *Quantum Dots*

   Quantum dots can be used for immunoassays for proteins and other analyses. This is important for characterizing the inner workings of biomedically-relevant processes.

3. *Magnetic Nanoparticles*

   A great example of magnetic nanoparticles used in biomedical applications is iron oxide nanoparticles. These can be used for imaging applications such as in magnetic resonance imaging (MRI) as the magnetic nanoparticles can be easily guided through the body with the application of an external magnetic field.

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4. *Carbon Nanotubes*

Carbon nanotubes (CNTs) such as single-walled carbon nanotubes and mutil-walled carbon nanotubes have excellent electrical and thermal conductivity. They are also extremely strong mechanically, and very stable chemically. To top this, they are also organic and therefore bio-compatible. They can therefore be used in a variety of biomedical applications as carriers or conductors.

Table 1 below summarizes this list of biomedically important classes of nanomaterials and their intrinsic properties that contribute to their unique biomedical applications in nano-medicinal therapy.

Table 1 Properties of Typical Nanomaterials in Biomedical Applications

Category	**Examples**	**Intrinsic Properties**	**Biomedical Applications**
Metal	Au, Ag	Surface Plasmon Resonance (SPR)	Bio-sensing, drug delivery, bio-imaging
Semiconductor/Quantum Dots	CdS, CdSe	Fluorescence, Luminescence	Immunoassays, bio-imaging, bio-sensing
Magnetic	Fe_3O_4	Magnetic attraction	MRI, drug delivery
Carbon	CNTs, Fullerene	Electronic and mechanical properties, chemical stability, superior conductivity	Drug and gene delivery, therapy, biosensing

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2 Choice of Ligands

The key advantage that nanomaterials bring to the table is their enlarged surface area. Thus surface modification is a key aspect in the use of nanomaterials in biomedical applications. Careful and selective surface treatment can meld the nanomaterial of choice for the right application, depending on whether the nanomaterial is to be used for analysis, sensing, imaging, or diagnostics.

As such, the choice of ligands that are to be attached to the nanomaterial surface are of utmost importance. A variety of different ligands may be attached to a nanomaterial depending on what effect is to be achieved. For example, ligands containing bulky hydrophobic groups may be attached to nanomaterial surfaces to prevent agglomeration. Conversely, two different ligands may be chosen to force nanomaterials to interact forming weak bonds between nanoparticles. This is known as ligand coupling.

Ligands may also be attached to act like “tags” for molecular recognition properties that can be exploited in drug targeting and in bio-imaging application as a marker. Ligands may also be attached to nanomaterial surface to define the properties of the nanomaterial itself. The smaller the nanomaterial, the larger the impact of the ligand on it. Multiple ligands may be coupled on to a nanomaterial too. Finally, ligands can be used to fix the polarity and consequently the solubility of the nanomaterial. If we want to precipitate a nanomaterial out of a hydrophilic solution, we simply functionalize it with a hydrophobic ligand and the nanomaterial will instantaneously precipitate out of the solution forming a cloud on the top.

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3 Nanomaterial Surface Functionalization

Surface functionalization of nanomaterials can be broadly classified into 2 categories:

1. *Non-covalent binding: Physisorption*

   This type of binding is fairly weak where the bonds between the ligand and the nanomaterial are through weak electrostatic interactions, hydrogen bonding, and/or hydrophobic interactions. This type of surface functionalization is useful when trying to form surface coatings for stabilizing individual nanoparticles in solution. It can also be used to inhibit agglomeration via steric repulsion of bulky ligand molecules in the surface coat.

2. *Covalent binding: Chemisorption*

   This type of binding, in contrast, is a lot stronger than the non-covalent bonds formed by physisorption. The majority of surface functionalization methods are based on covalent bond formation. This technique offers a stronger bond which allows the ligand to be more stable on the surface making the linkage quite robust. Functional groups present on the ligand react with the substrate material and chemisorb to the nanomaterial surface to yield self-assembled structures. Typical examples of chemisorption include thiol/disulfide on metals (Au, Ag, Cu) and semiconductors (CdS, CdSe, ZnS), silanes on oxides (SiO_2, TiO_2), and phosphates on metal oxides (iron oxide, TiO_2). Of all the chemisorbed self-assembly systems, thiol/Au is the most studied and used.

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In the case of chemisorption, the nanomaterial is already pre-derivatized with a functional group. This group then reacts with the ligand that possesses complementary functional group through a ligand-exchange technique. In this case, the ligand should have at least equal or higher affinity than the capping molecule towards the nanomaterial. The other alternative is the two functional groups react together and form a derivatized final molecule as a result of the chemical synthesis. Table 2 shows a list of common complementary functional groups used for coupling ligands to nanomaterials and the resulting groups from the chemisorption.

Figure 1 diagrammatically shows the two broad types of surface functionalization techniques that can be applied to nanomaterials. The non-covalent (physical) bond is weak and therefore temporary, while the covalent (chemical) bond is strong and therefore more permanent.

4 Photo-Initiated Coupling Chemistry

Another method of surface modification for binding complex molecules like carbohydrates is known as photo-initiated coupling chemistry. Since carbohydrates are complex in structure, they are hard to chemically derivatize by combining one or more functional groups. But carbohydrates are very important and useful biomolecules to bind to nanomaterials for the formation of biomedical sensors. Glycoproteins and glycolipids are naturally occurring carbohydrates and are present at the surface of almost all living cells. Thus there is no easy and direct way to attach carbohydrates to a nanoparticle.

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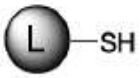
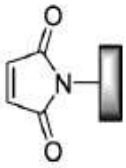
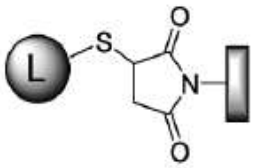
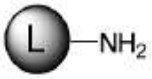
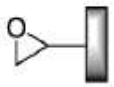
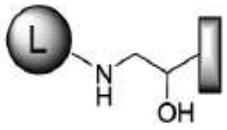
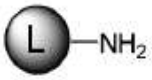
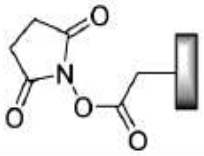
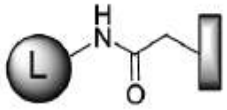
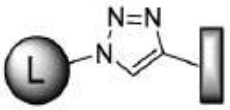
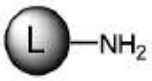
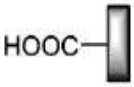
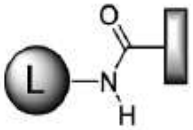
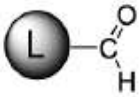
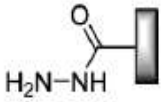
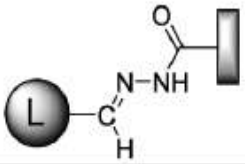
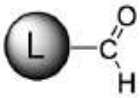
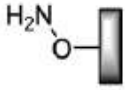
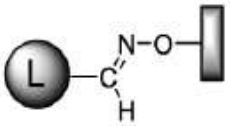
Table 2 Typical Complementary Functional Groups used in Chemisorption. Table from [1].

Ligand	Substrate	Ligand decorated surface	Ref.
			(76, 77)
			(78, 79)
			(80)
			(81, 82)
			(83)
			(84)
			(84, 85)

The alternative is to functionalize nanoparticles with a helper molecule known as perfluorophenyl azides (PFPAs) to help attach complex molecules and materials like carbohydrates to solid substrate surfaces. The azide component of the PFPA is exposed to light which then converts to a highly reactive nitrene group that inserts itself into CH and

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NH bonds creating very good covalent bonds. This chemistry is known photo-initiated coupling chemistry.

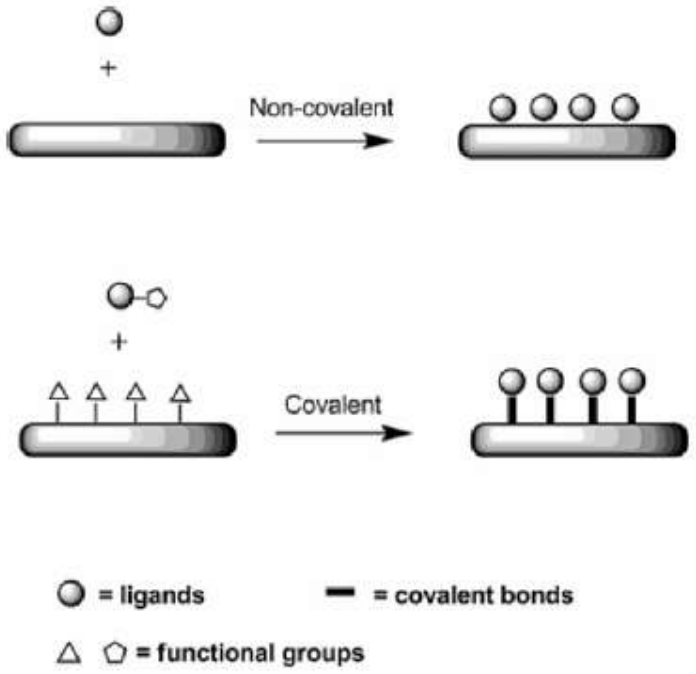

Figure 1 Surface modification of nanomaterials using non-covalent and covalent approaches. Figure from [1].

PFPA photocoupling chemistry is well-understood and well-established and can be used to attach carbohydrates to nanoparticles. This works by preparing PFPA-functionalized nanoparticles that can be subsequently used to covalently couple carbohydrate structures thanks to the highly reactive photo-activated nitrene species from the azide moiety of the PFPA. This coupling reaction is quite fast and often takes place in a few minutes.

Nanoparticles can be functionalized with the photocoupling agent (PFPA) by chemisorption via a simple solution incubation process. Gold nanoparticles can be easily functionalized Monosaccharides and oligosaccharides can be attached on to gold and iron oxide particles

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in this manner. A similar technique can also be used for attaching polymers too on to silica nanoparticles. The coated nanoparticles are stable in solution and are readily dispersed in water to give homogenous solutions.

Figure 2 below shows a schematic illustration of a gold nanoparticle that was successfully prepared using the PFPA photo-coupling chemistry outlined above. Monosaccharides were chosen since these are the smallest carbohydrate structures. They are also the most challenging for the photo-coupling chemistry detailed above since they have the least amount of affinity as a ligand compared to oligosaccharides. This is because the probability of attaching the ligand by a simple C-H insertion reaction increases as the size of the carbohydrate structure, i.e. the number of C-H bonds, increases.

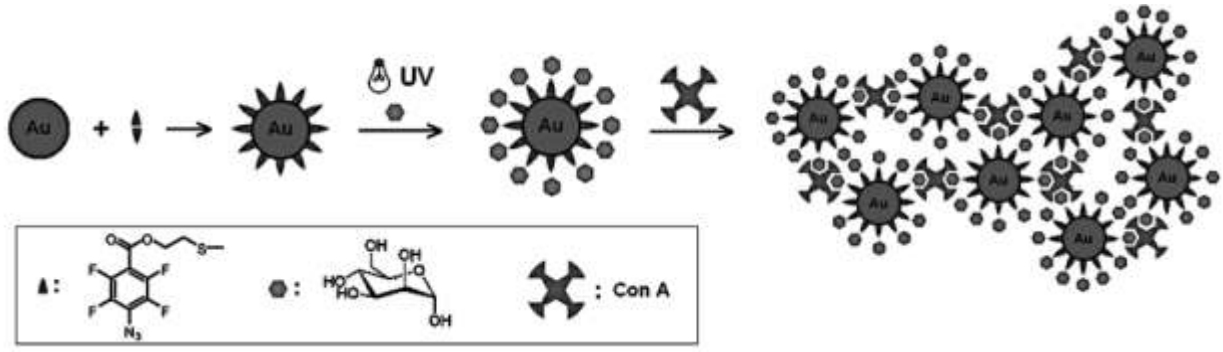

Figure 2 Illustration of Gold Nanoparticles with *Con A* and Formation of gold nanoparticle aggregates. Figure from [1]

5 Synthesis of Surface Functionalized Gold Nanoparticles

A single process to both prepare gold nanoparticles and functionalize them with PFPAs was developed in [1]. These gold nanoparticles were 20nm in diameter. Once these surface-functionalized carbohydrates were formulated, a covalent attachment of carbohydrate ligand was performed by activating the azide group in PFPA using UV light. The surface

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coverage, measured using thermal gravimetric analysis (TGA), was around 80% which is pretty substantial.

These coated gold nanoparticles were then developed into calorimetric biosensor for probing carbohydrate-protein interactions. For example, when these gold nanoparticles were treated with the *Con A* target protein, the absorbance spectrum of the nanoparticles would broaden and be red-shifted which could be easily detected with modern computer software. The gold nanoparticles were originally wine-red in color as can be seen through surface plasmon resonance (SPR) with an absorbance peak at 520nm. Upon surface functionalization, the nanoparticles form cross-linked aggregates as can be seen in Figure 3 where the *Con A* protein acts as the cross-linker.

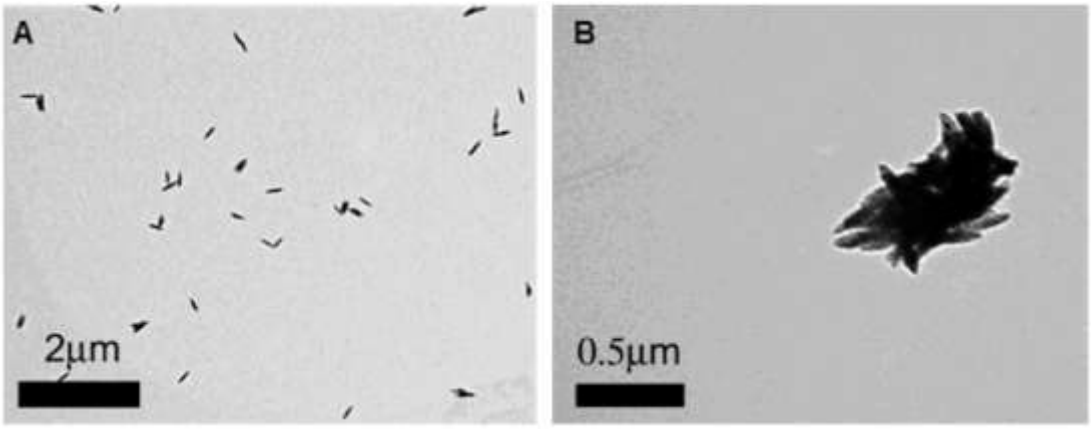

Figure 3 TEM Images of resulting NP aggregation after treating carbohydrate-functionalized Au NPs with *Con A*. Figure from [1].

This formation of aggregates is manifested as a red-shift in their SPR absorption and a broadening of the peak as can be seen in Figure 4, resulting in a change in colour of the solution. Of course, the extent to which this colour change is detectable depends on the surface coverage of carbohydrate as well as the extent of nanoparticle agglomeration.

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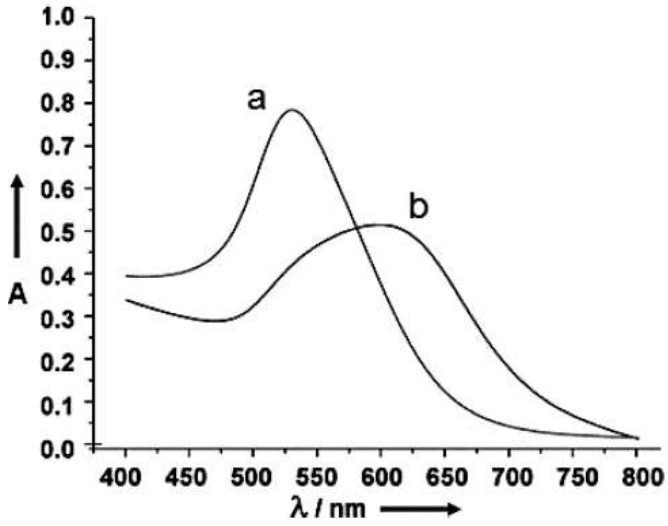

Figure 4 UV-Vis absorption spectra of (a) Pristine Au NPs with surface-coupled D-mannose monosaccharide and (b) after the Au NPs are treating with *Con A* protein. Figure from [1].

The specificity of this biosensor was testing by treating the same monosaccharide-functionalized Au NPs with other proteins, but the colour of the resulting solutions remain unchanged and no notable red-shifts of the SPR peak were observed. This thus demonstrates the high selectivity of this advanced glyconanoparticle-based sensing system.

6 Summary

Since surfaces are at the boundary of every material, they form as the interface between the nanomaterial and the physical phase surrounding the nanomaterial. The surface also plays a vital role in the properties and functions of nanomaterials. Also, the smaller the material, the more critical the role played by the surface as compared to the bulk. Nanomaterials, owing to their size, therefore have high surface energy and as such any ligands present on the surface can serve to act as points of communication with external receptors and detectors. The ligands must therefore be carefully chosen since they play a variety of roles

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including determining surface and nanomaterial physical properties, inhibiting or encouraging agglomeration, and helping to translate various molecular events into electrical signals that can be reliably detected externally.

Effective surface coupling chemistry is of utmost importance. Consequently, novel techniques of attaching complex molecules on to nanomaterials are always in demand. These techniques need to be able to accommodate differences in ligands and their bioaffinities and at the same time also be able to result in surfaces that are not only bioactive but also stable.

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